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WPI Abstract Accession No. 85-237601/39 & DE 3409372 A WPI Abstract Accession No. 85-155917/26 & JP 600087225 A BIULL EKSP BIOL MED (USSR), Vol. 97, No. 7, 1981, 68-70 & MEDLINE Abstract Accession No. 82047152

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(54) Protein/oxidised regenerated cellulose complexes

(57) There is disclosed materials such as powders, films, flakes or sponges comprising a protein complexed with oxidised regenerated cellulose (ORC). The preferred protein is collagen. Processes for the preparation of the complexes comprise dispersing or dissolving protein in aqueous solvent, adding soluble or insoluble ORC, followed by removal of the solvent. The complexes are used especially for wound dressings and the like, and exhibit useful binding to growth factors and matrix metalloproteinases.

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FIG. 1

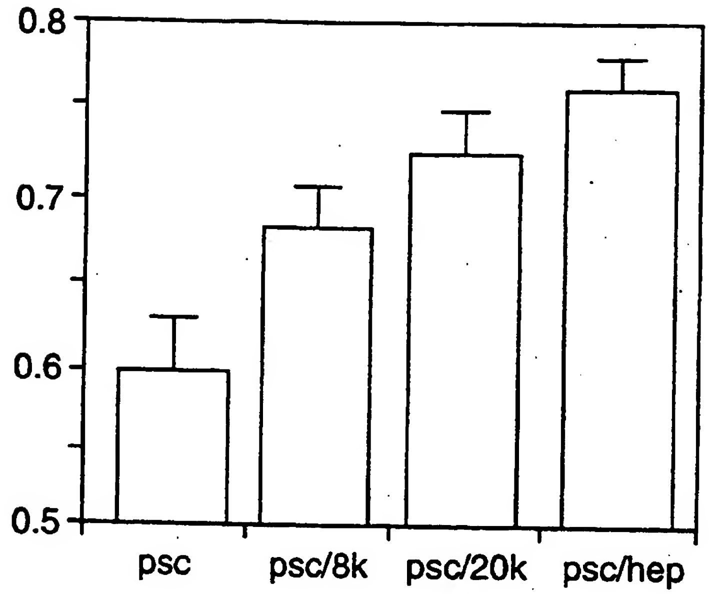

0.8
0.7
0.6
0.5
psc
psc/8k
psc/20k
psc/hep

FIG. 3

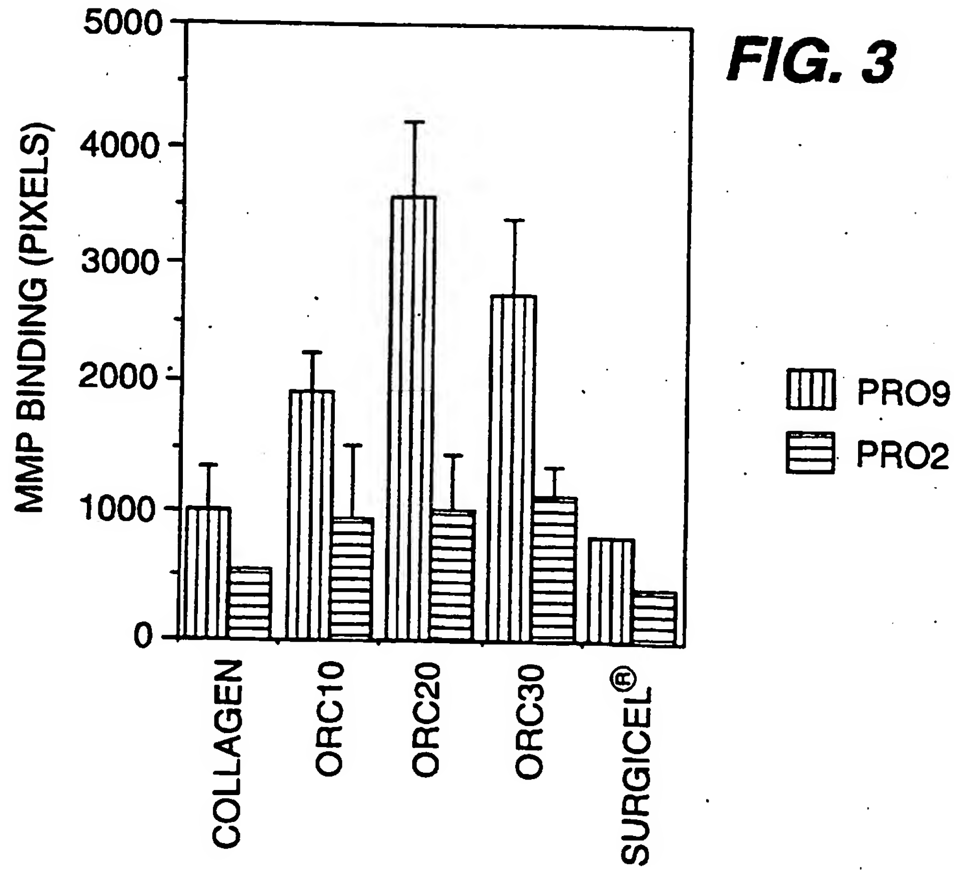
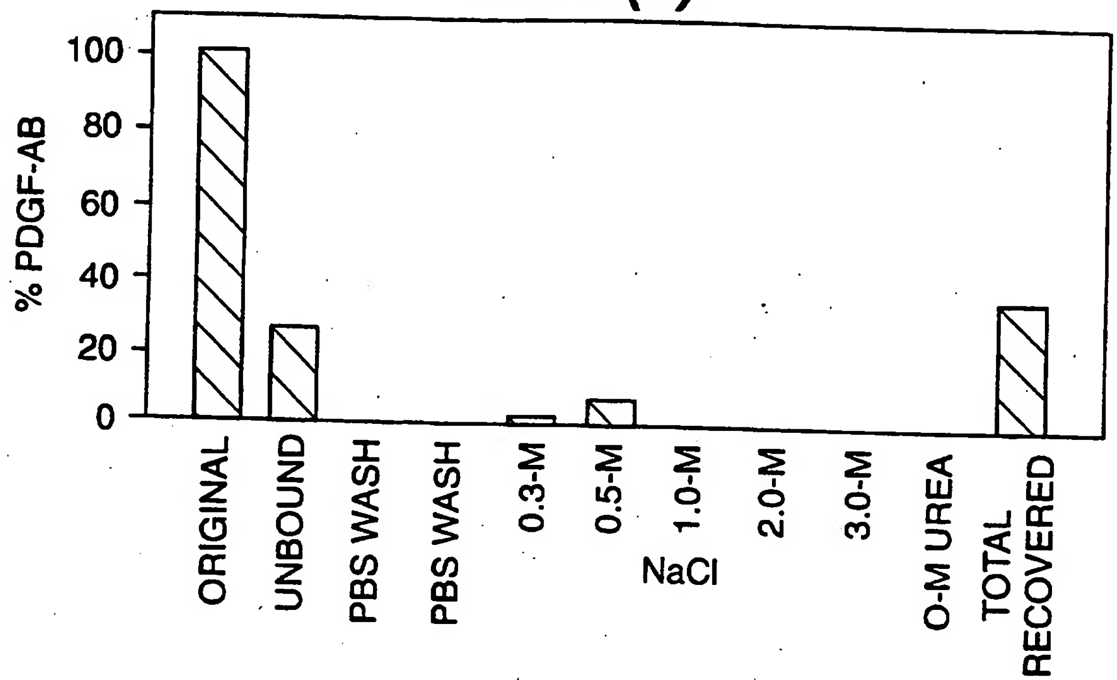
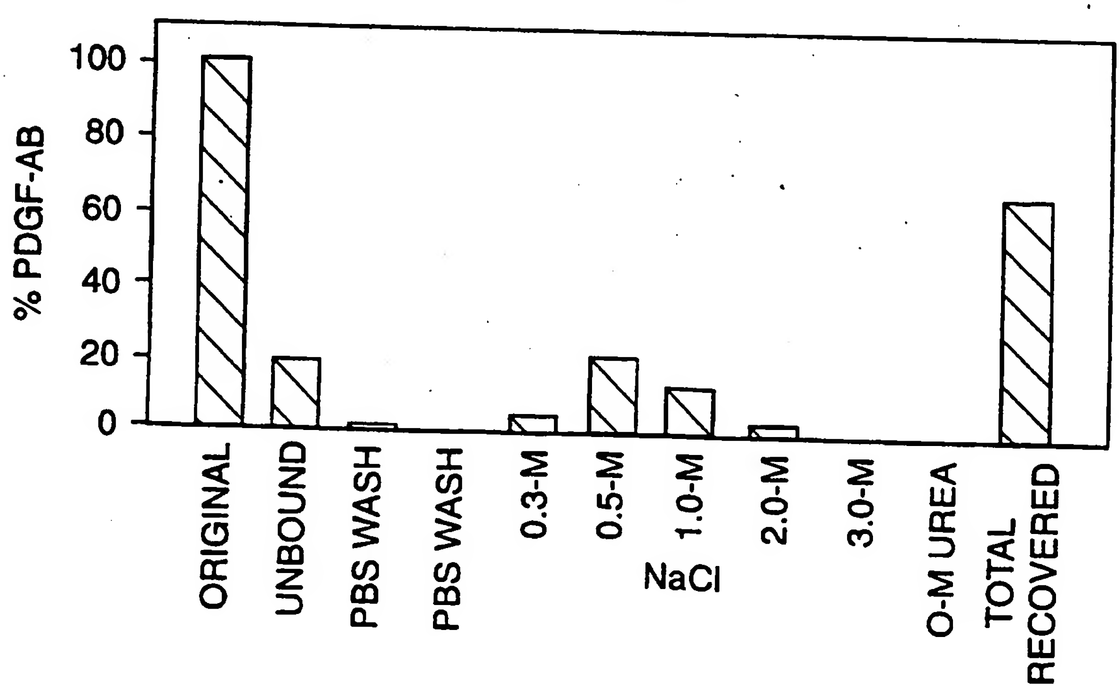

5000
4000
3000
2000
1000
0
MMP BINDING (PIXELS)
COLLAGEN
ORC10
ORC20
ORC30
SURGICEL®
PRO9
PRO2

FIG. 2(a)



100
80
60
40
20
0
% PDGF-AB
ORIGINAL
UNBOUND
PBS WASH
PBS WASH
0.3-M
0.5-M
1.0-M
2.0-M
3.0-M
NaCl
O-M UREA
TOTAL RECOVERED

FIG. 2(b)



100
80
60
40
20
0
% PDGF-AB
ORIGINAL
UNBOUND
PBS WASH
PBS WASH
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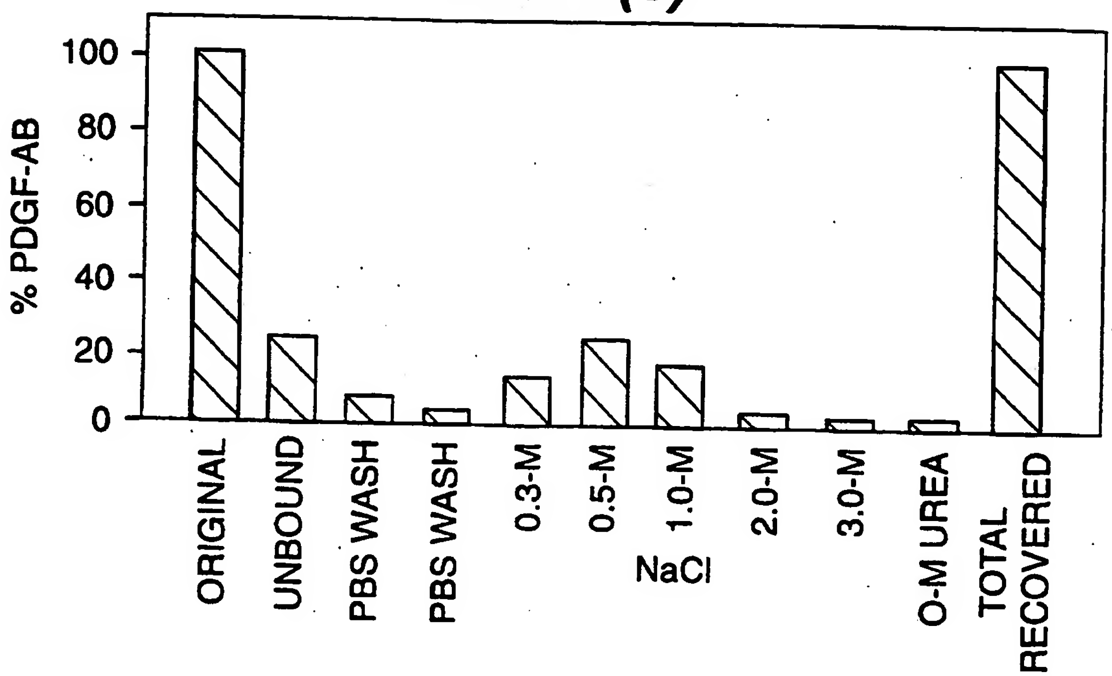
1.0-M
2.0-M
3.0-M
NaCl
O-M UREA
TOTAL RECOVERED

FIG. 2(c)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PBS WASH
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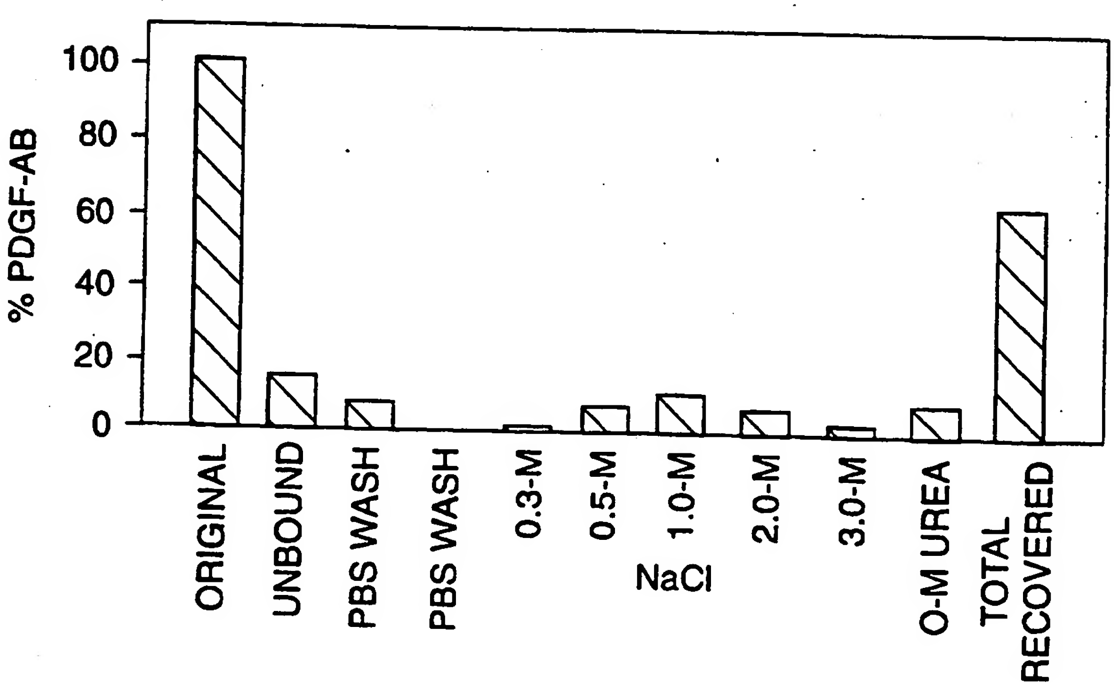
2.0-M
3.0-M
NaCl
O-M UREA
TOTAL RECOVERED

FIG. 2(d)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PBS WASH
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0.5-M
1.0-M
2.0-M
3.0-M
NaCl
O-M UREA
TOTAL RECOVERED

PROTEIN/OXIDIZED REGENERATED CELLULOSE COMPLEXES

The present invention relates to complexes of structural proteins such as collagen with oxidized regenerated cellulose, processes for the preparation of such complexes, and uses of such complexes.

Collagen, which is a structural protein of animal origin, is known in various forms for use as a wound dressing material. Likewise, naturally occurring and chemically modified polysaccharides such as alginates, starch derivatives and oxidised regenerated cellulose (ORC) are known for use in wound dressings and for other bio-medical applications.

GB-A-1515963 describes cross-linked collagen-mucopolysaccharide composite materials for use in medical and surgical applications, blood vessel grafts and all forms of surgical prostheses. The composite material contains at least 0.5% by weight of a mucopolysaccharide irreversibly bound to collagen. The mucopolysaccharide is an animal polysaccharide containing hexosamine residues, such as hyaluronic acid, chondroitin sulphate or heparin sulphate. The composite materials are said to exhibit greater resistance to resorption and better blood compatibility than simple collagen materials.

US-A-4614794 describes complexes formed between collagen and polyanionic plant polysaccharides, such as sodium alginate. The complexes are preferably formed by combining the protein and the polysaccharide at a pH which is no higher than the isoelectric point of the protein. The resulting complexes are said to be suitable for a wide variety of medical and surgical applications, including wound dressings. There is no disclosure of the use of oxidised regenerated cellulose as the polyanionic plant polysaccharide. The specification also teaches that proteins other than collagen, such as fibrin or elastin may

be used in the formation of useful protein/polysaccharide complexes.

The above-described collagen, polysaccharide and collagen/polysaccharide would dressing materials provide important advantages. The materials are of natural, biological origin (albeit sometimes chemically modified), and consequently tend to have low antigenicity. The materials are generally bio-absorbable, which reduces the trauma associated with removal of conventional wound dressing materials from the surface of the wound. Furthermore, some of these materials can have positive therapeutic effects on wound healing. For example, some animal mucopolysaccharides such as hyaluronic acid are thought to exert a chemotactic effect on wound healing cells such as fibroblasts, and thereby promote the growth and development of such cells. Nevertheless, there remains a need for improved wound dressing materials of this general type exhibiting still better control of physical properties and biological absorption rates, still better therapeutic effects on wound healing, and reduced cost. The present invention addresses these technical issues, and further provides related advantages.

The present invention provides a material comprising a protein complexed with oxidised regenerated cellulose.

Preferably, the protein and oxidised regenerated cellulose together make up at least 75% by weight of the material, more preferably at least 90% by weight of the material. Other components of the material may include 0-25% by weight of one or more other biocompatible polysaccharides, for example alginates such as sodium alginate or calcium alginate, starch derivatives such as sodium starch glycolate, cellulose derivatives such as methyl cellulose or carboxymethyl cellulose, or glycosaminoglycans such as hyaluronic acid or its salts, chondroitin sulphate or heparan sulphate. The material may

also comprise up to 20% by weight, preferably up to 10% by weight of water. The material may also contain 0-40% by weight, preferably 0-25% by weight of a plasticiser such as glycerol. The material may also comprise 0-10% by weight, preferably 0-5% by weight of one or more therapeutic wound healing agents, such as non-steroidal anti-inflammatory drugs (eg. acetaminophen), steroids, antibiotics (eg. penicillins or streptomycins), antiseptics (eg. silver sulfadiazine or chlorhexidine), or growth factors (eg. fibroblast growth factor or platelet derived growth factor).

Preferably, the weight ratio of protein to oxidised regenerated cellulose (ORC) is from 1:99.99 to 99.99:1. More preferably, the weight ratio is in the range 2:1 to 99.9:1, still more preferably it is in the range 2:1 to 95:1.

The material according to the present invention may be in any convenient form, such as a powder, microspheres, flakes, a mat or a film. However, preferably, the material according to the present invention is in the form of a freeze-dried or solvent-dried sponge. Preferably, the pore size in the sponge is in the region of 10-500μm.

Suitable proteins for the materials according to the present invention include the structural proteins such as fibronectin, fibrin, laminin, elastin and collagen. Preferably the protein comprises collagen, and more preferably it consists essentially of collagen. The collagen may be collagen obtained from any natural source, including microbiological sources, but is preferably collagen obtained from bovine corium that has been rendered largely free of non-collagenous components, for example fat, non-collagenous proteins, polysaccharides and other carbohydrates as described in US Patents Nos. 4614794 and 4320201, the entire contents of which are hereby incorporated by reference. The collagen may also be chemically modified collagen, for example an atelocollagen

obtained by removing the immunogenic telopeptides from natural collagen. The collagen may also comprise solubilised collagen or soluble collagen fragments having molecular weights in the range 5,000-100,000, preferably 5,000-50,000 obtained by pepsin treatment of natural collagen in known fashion.

The oxidised regenerated cellulose (ORC) can be obtained by the process described in US Patent No. 3122479, the entire content of which is incorporated herein by reference. This material offers numerous advantages including the features that it is biocompatible, biodegradable, non-immunogenic and readily commercially available. ORC is available with varying degrees of oxidation and hence rates of degradation. This material has a proven medical history with applications as both a haemostat (under the Registered Trade Mark SURGICEL) and anti-adhesion barrier during surgery (Registered Trade Mark INTERCEED). The ORC may be used in the form of insoluble fibers, including woven, non-woven and knitted fabrics. In other preferred embodiments, the ORC is in the form of water-soluble low molecular weight fragments obtained by alkali hydrolysis of ORC.

Surprisingly, despite the widespread study of collagen and ORC separately in the wound treatment medical art, complexes according to the present invention are new. The ready availability of both collagen and ORC having a range of controllable properties means that the properties of the materials according to the present invention can be controlled to an exceptional degree. The materials according to the invention may be used as haemostats, for tissue replacement, topical wound dressings, for periodontal guided tissue regeneration, to deliver drugs, and for related purposes.

In particular, the rate of biological absorption, porosity and density of the materials can be controlled. This is one important advantage of the materials according

to the present invention. This advantage makes the materials particularly suitable for use in wound dressings, and accordingly the present invention also provides the use of a material according to the invention for the preparation of a dressing for the treatment of wounds.

The complexes according to the present invention are especially suitable for the preparation of pharmaceutical compositions including wound dressings for the treatment of chronic wounds and other medical conditions mediated by matrix metalloproteinases (MMP's). This is because the complexes exhibit enhanced binding of MMP's relative to either collagen or ORC alone.

It has also been found, surprisingly, that the materials according to the present invention have an excellent ability to bind to growth factors, in particular, platelet derived growth factor. Accordingly, the present invention also provides the use of a material according to the invention to bind one or more cell growth factors. Preferably, the cell growth factor is platelet derived cell growth factor (PDGF).

The present invention further provides a method of separating cell growth factors from a biological sample or organism, the method comprising:

(i) containing the biological sample or organism with a material according to the present invention, the contacting being carried out *in vivo* or *in vitro*, to bind the growth factors to the material; and

(ii) recovering the bound growth factors from the material.

The present invention further provides a method of preparing an active wound dressing material comprising the steps of:

(i) contacting a material according to the present invention with a biological medium containing cell growth factors to bind the cell growth factors to the material;

(ii) washing and drying the material having the cell growth factors bound thereto to form said active wound dressing material. Preferably, the cell growth factors comprise platelet derived growth factor.

Finally, the present invention provides a process for the preparation of a material according to the present invention, the process comprising the steps of:

providing an aqueous dispersion of a protein; and/immersing or dispersing oxidised regenerated cellulose in the aqueous dispersion; following by removing water from the aqueous dispersion to leave a material comprising protein complexed with oxidised regenerated cellulose.

Preferably, the oxidised regenerated cellulose and protein are as described above for the preferred embodiments of the materials according to the present invention. The optional, additional components in the materials according to the present invention are preferably included in the aqueous dispersion prior to removal of water from the aqueous dispersion.

The water can be removed from the aqueous dispersion by evaporation, for example by evaporation from the dispersion in a tray to leave a film of material. However, preferably, the water is removed by freeze-drying (lyophilizing) or solvent-drying to produce the material in the form of a sponge. Preferably, the aqueous dispersion contains 5-30mg/ml of collagen.

Preferably, the process according to the present invention further comprises treating the protein and polysaccharide in the dispersion, or in the material, with a cross-linking agent such as carbodiimide, hexamethylene diisocyanate (HMDI) or glutaraldehyde. Alternatively, cross-linking may be carried out dehydrothermally. The method of cross-linking can markedly affect the final product. For example, HMDI cross-links the primary amino

groups on the protein within the complex, whereas carbodiimide cross-links carbohydrate on the ORC to primary amino groups on the protein.

Preferably, the pH of the dispersion is adjusted to pH 3-4.5. This pH range is less than the isoelectric pH of collagen.

The oxidised regenerated cellulose may be added to the aqueous dispersion of protein in the form of a suspension or solution of the oxidised regenerated cellulose, preferably at a comparable pH to the collagen suspension, following by mixing by stirring or homogenisation. Alternatively, dry fibers or fabric of oxidised regenerated cellulose may be immersed in the aqueous dispersion of collagen.

Specific embodiments of the present invention will now be described further, by way of example, with reference to the accompanying drawings, in which:

Figure 1 shows a graph of fibroblast cell growth (in arbitrary units) on serum treated films consisting of (a) pepsin solubilised collagen, (b) pepsin solubilised collagen complexed with ORC fragments having average molecular weight 8,000, (c) pepsin solubilised collagen complexed with ORC fragments having average molecular weight about 20,000, and (d) pepsin solubilised collagen complexed with heparan sulphate (for comparison);

Figure 2 shows a graph of percentage binding of platelet derived growth factor (PDGF) to the following materials: (a) plastics, (b) collagen sponge, (c) collagen/solubilised ORC sponge, (d) Interceed® ORC, and the ease of removal of this growth factor from the materials; and

Figure 3 shows a graph of relative amounts of MMP binding measured for a collagen sponge and Surgicel® ORC fabric (comparative measurements) and for sponges of collagen complexed with 10 wt.%, 20 wt.% and 30 wt.% of fibrous ORC.

Example 1: Preparation of a collagen/fibrous ORC sponge

Lyophilised collagen, prepared as described in US Patent No. 4614794 or 4320201, is re-suspended in cold 0.05M acetic acid at a concentration of 10mg/ml. Milled ORC powder (milled Surgicel® cloth) is added to the suspension in a ratio of 1:3 ORC: collagen and homogenised using a Waring Blendor on low speed for 3 x 30s. The complex suspension is degassed in a vacuum oven for 10 min. and then poured to a depth of 3mm and blast frozen. The frozen suspension is then either freeze-dried and dehydrothermally cross-linked using a programmable Edwards freeze-drier with a temperature ramping facility, or dried using a solvent drying process as described in US-A-3157524.

Example 2: Preparation of a collagen/ORC oligosaccharide sponge

Soluble collagen is prepared by the published method of E.J. Miller and R.K. Rhodes "Preparation and Characterisation of the Different Types of Collagen", Methods Enzymol Vol. 82, pages 33-64 (1982). Soluble oligosaccharides of ORC are prepared as described in a co-pending Patent Application filed on the same date as this application and commonly assigned herewith. Briefly, the ORC oligosaccharides are prepared by treating commercially available ORC with 6M sodium hydroxide solution at 37°C for 45 minutes, followed by neutralisation and dialysis to remove fragments and impurities having molecular weight below 1000. The resulting soluble oligosaccharides of ORC are slowly added while stirring to soluble collagen (0.75mg/ml) (both in cold 0.05M acetic acid) until no further precipitation of the complex occurs. The complex precipitate is isolated by centrifugation, washed in phosphate buffer at pH 7.2, and re-suspended, by homogenisation, at 30% w/v in the same buffer. The suspension is poured to a depth of 3mm, blast frozen at -30°C and freeze-dried.

Example 3: Preparation of a collagen/ORC film

A collagen/ORC film is made by the methods described in either of the first two examples, except that the suspensions are not precipitated but air-dried rather than frozen and freeze-dried. To prepare flexible films a small amount of glycerol may be added to the suspensions.

Example 4: Preparation of a collagen sponge/ORC fabric composite

A collagen suspension is prepared as described in Example 1 and poured to a depth of 2-3mm over a sheet of Surgicel® fabric in a mould. The mixture is then frozen and freeze-dried as previously described.

Example 5: Preparation of a collagen film/ORC fabric composite

A collagen suspension is prepared as described in Example 1 and poured to a depth of 2-3mm over a sheet of Surgicel® fabric in a mould. The mixture is then air-dried.

Example 6: Preparation of collagen/ORC sponge using a pre-gelling process of the ORC

ORC fabric (Surgicel®) is suspended (4% w/v) in dilute alkali ($NaHCO_3$) at pH 8.0 for a time that is sufficient to convert the fabric to a gelatinous mass. Collagen slurry is added at the same pH to give a final solids content of both Surgicel® and collagen of 1% w/v. The slurry is stirred and the pH adjusted to pH 3.0-4.0 using acetic acid. The final slurry is moulded, frozen and freeze-dried under vacuum.

The advantageous properties of the materials according to the present invention prepared as above were determined as follows:

Procedure 1: Promotion of fibroblast cell growth

A collagen/alkali-soluble ORC complex film was prepared in a petri dish as described in Examples 2/3, and serum poured over the film and incubated at 37°C overnight. The

serum was removed and the effects on fibroblast cell growth measured (Fig. 1). Cell growth was observed for a pepsin solubilised collagen (PSC) film (control), PSC plus ORC oligosaccharides having average molecular weights of about 8,000 and about 20,000 prepared as described above, and PSC plus heparin was included as a positive control. The results show that the collagen/ORC fragments film appears to bind factors from the serum which stimulate cell growth.

Procedure 2: Binding of platelet derived growth factor

PDGF binding studies were carried out as follows: Small sections of test material (approximately 1cm^2 squares of Interceed® ORC fabric, and approximately 1cm x 0.5cm x 0.4cm sections of collagen sponge) were weighed and soaked in 100mM sodium phosphate dibasic buffer containing 150mM sodium chloride (total volume 1ml) for at least one hour at room temperature. Samples were then incubated with 2% bovine serum albumin (BSA) in phosphate buffered saline (PBS) for 2 hours at room temperature. 22ng of PDGF was then added to each sample in 250μl of PBS containing 2% BSA, and samples were then incubated for a further hour at 37°C. Each sample was then washed three times with 250μl PBS, followed by increasing concentrations of sodium chloride. Finally, each sample was washed with 4.0M urea. PDGF ELISA analyses of the original PDGF preparation and the various washings were carried out. The data shown in Fig. 2 indicate that the growth factor can be totally recovered from the composite of collagen and ORC while the individual components appear not to release all the growth factor. The binding characteristics are also uniquely different for the collagen/ORC complex compared with the individual components. These observations indicate that the complex has unique binding of PDGF which may be utilised appropriately for both exogenous binding and endogenous binding and release of growth factor.

Procedure 3: Haemostasis

Recently weaned, female, cross-bred swine, in the

approximate weight range of 22-45kg, were anaesthetised with Isoflurane (Aerrane®). A surgical plane of anaesthesia was achieved and demonstrated by a null response to a noxious stimulus. While under anaesthesia, physiological parameters such as temperature, pulse and respiration were monitored and documented.

Animals were placed in dorsal recumbency with all limbs secured. The abdominal cavity was opened along the midline. The spleen was exteriorized. Haemostasis incision wounds were made using a scalpel on the surface of the spleen. Wound lengths were controlled and ranged from 0.5 to 2.0cm. Wound depth was controlled and ranged from approximately 1.5 to 3.0mm deep. The depth of each wound was kept constant by marking the scalpel blade at the appropriate depth. The length of the incision was controlled by using a suitable template which had been clearly marked for the appropriate incision length. The first wound at the distal end of the spleen served as a negative control and was permitted to bleed for twelve minutes to demonstrate the bleeding potential of an untreated wound. The second wound was made approximately 1.0cm proximal to the first incision. This and subsequent incisions were used as test incisions. A final incision was used as a termination negative control to demonstrate that the bleeding potential of an untreated wound did not change.

After incisions were created, a stop-watch was started and gauze (negative control), collagen, collagen/ORC composites and ORC (under gauze) were quickly applied to the wounds. Gentle pressure was applied to the top surface of the gauze for 2 minutes and then the pressure was released. This procedure was repeated at 30 second intervals until the haemorrhage was controlled. Control of the haemorrhage (haemostasis) was defined as no renewed bleeding for 30 seconds. The time of the last release of pressure was the time to achieve haemostasis. The order in which the test or control articles were placed on the wounds was assigned by

computerised randomisation.

Animals were euthanized by I.V. injection with a commercially available solution or other suitable means before recovering from anaesthesia.

These studies show that composite materials of collagen and ORC achieve more rapid haemostasis than either collagen or ORC alone.

Procedure 4: Adhesion Prevention

Collagen/ORC composite materials, as described in the preceding examples, were tested against collagen material and Interceed® alone, for their ability to prevent adhesions in a Rabbit Uterine Horn Model.

Female New Zealand White rabbits between 2.5 and 3.0kg were anaesthetised by an intramuscular injection of a combination of ketamine hydrochloride and xylazine (5mg/kg + 34mg/kg) anaesthetic at a dose of 0.6ml/kg. The animals were placed in dorsal recumbency and the entire abdominal ~~area clipped free of fur~~. The surgical site was scrubbed with povidone iodine soap, wiped with alcohol, painted with povidone iodine solution and routinely draped. The animals were placed on halothane/oxygen inhalation for maintenance of anaesthesia.

Incisions (approximately 3cm) were made in the caudal ventral midline into the peritoneal cavity and the uterine horns exteriorized.

The middle third segment of the serosa of both uterine horns were abraded using the sharp edge of a No. 10 scalpel blade. A new blade was used for each animal. The affected areas were approximately 1cm from the uterine bifurcation for a length of 5cm. Haemostasis was achieved by digital pressure and application of a sterile gauze.

Prior to surgery, treatments were randomised using a random number table to determine which treatment each animal received. The surgeon was blinded to the scheme. When the abrasion procedure was complete an assistant gave the test material to the surgeon for application. Test materials and controls were applied to the abraded uterine horns.

Animals were returned to their individual cages and observed to recover. At 14 days after surgery, the animals were weighed and then euthanized with an intravenous injection of a sodium pentobarbital based solution. The peritoneal cavities were opened and the viscera examined for adverse changes. Evaluation of the extent of adhesions present on the uterine horns was subjective using a scoring system of 0 to 3, where 0 = no adhesions, 1 = 21-25%, 2 = 26-50% and 3 = >50% adhesion involvement.

These studies show that composite materials of collagen and ORC are better at reducing the occurrence of adhesions than either collagen or ORC alone.

Procedure 5: Matrix Metalloproteinase Binding

The effect of complexation between collagen and ORC on matrix matalloproteinase (MMP) binding was assessed as follows.

Collagen/fibrous ORC sponges containing 10%, 20% and 30% by weight of fibrous ORC were prepared by the procedure described in Example 1. An ORC-free collagen sponge was prepared for comparison purposes. A sample of Surgicel® ORC fabric was also prepared for comparison.

Briefly 50mg of each material was placed in a 15ml plastic beaker containing 2.5ml of an acute wound fluid diluted to 1:50 in a proteolysis buffer (50mM tris/HCL pH7.8, 50mM $CaCl_2$, 0.5MNaCl) and incubated at 37°C on a shaking water bath for 3 hours. Acute wound fluid contains various proteinases, including matrix metalloproteinases and

many of these enzymes will preferentially bind to various dressing materials. The excess fluid absorbed by each material was mechanically expressed using a metal spatula and discarded. The remaining dressings were placed into pre-packed 2ml syringes (each syringe contained 0.5ml volume of 2.5mm glass beads). 4ml of proteolysis buffer was forced through the syringe in 1ml aliquots which were discarded. At this washing stage all of the unbound proteinases and proteinaseses which were only weakly bound to the dressing material had been removed from the dressing leaving the more tightly bound forms. The buffer rinsed dressing were then removed to another 15ml plastic beaker. 1ml of non-denaturing sample buffer (6.3ml 0.05M tris/HCL pH6.8, 2.5ml glycerol, 0.5g SDS, 16.2ml water and bromophenol blue) was added to each sample which were placed on an orbital shakier at setting six for 2 hours. The sample buffer detaches the tightly bound proteinases from the materials which are then present in the sample buffer itself. After this time 20 microlitres of sample buffer was taken from each container and subjected to gelatin substrate SDS-polyacrylamide gel electrophoresis (zymography), as described by Heussen C. and Dowdle E.B., Anal. Biochem. 102:196-202 (1980).

The area of the individual zones of clearance on the gels, which are due to proteinase activity, were accurately measured by the Optilab system. This was achieved by repeating each binding experiment (n=3) and analysing the results statistically by the Students T test, where $P \leq 0.05$. Analysis was against controls of pure collagen.

The results shown in Figure 3 demonstrate a surprising synergistic improvement in MMP binding for the complexes of collagen with ORC. Data are presented for the proenzyme forms (PRO2 and PRO9) of matrix metalloproteinase 2 (Gelatinase A) and matrix metalloproteinase 9 (Gelatinase B). Without wishing to be bound by any theory, it is thought that the improvement may be related to neutralization of opposing electrostatic charges on the

collagen and the ORC by complexation.

The above examples are intended for the purpose of illustration only. Many other embodiments falling within the scope of the accompanying claims will be apparent to the skilled reader.

CLAIMS

1. A material comprising protein complexed with oxidised regenerated cellulose.

2. A material according to claim 1, wherein the protein and oxidised regenerated cellulose together make up at least 75% by weight of the material.

3. A material according to claim 2, wherein the protein and oxidised regenerated cellulose together make up at least 90% by weight of the material.

4. A material according to claim 1, 2 or 3, wherein the material is a sponge.

5. A material according to claim 1, 2 or 3, wherein the material is a solid film.

6. A material according to any preceding claim, wherein the weight ratio of protein to oxidised regenerated cellulose is from 1:99.99 to 99.99:1.

7. A material according to claim 6, wherein the weight ratio of protein to oxidised regenerated cellulose is in the range 2:1 to 99.9:1.

8. A material according to any preceding claim, wherein the protein comprises collagen, fibronectin, fibrin, laminin or elastin.

9. A material according to any preceding claim, wherein the protein consists essentially of collagen.

10. A material according to any proceeding claim, wherein the protein comprises partially hydrolysed, soluble collagen having molecular weights in the range 5,000-100,000.

11. A material according to any of claims 1 to 9, wherein the collagen is fibrous, substantially insoluble collagen.

12. A material according to any preceding claim, where in the oxidised regenerated cellulose comprises water-soluble oxidised regenerated cellulose fragments having molecular weights in the range 5,000-50,000.

13. A material according to any of claims 1 to 11 wherein the oxidised regenerated cellulose is fibrous and substantially insoluble in water.

14. A material according to claim 13, wherein the oxidised regenerated cellulose is in the form of a knitted, woven or non-woven fabric.

15. Use of a material according to any preceding claim for the preparation of a dressing for the treatment of wounds.

16. Use of a material according to any of claims 1 to 14 for the preparation of a composition for the prevention of surgical adhesion.

17. Use of a material according to any of claims 1 to 14 for the preparation of a composition for the treatment of medical conditions mediated by a matrix metalloproteinase.

18. Use according to claim 17, wherein the medical conditions include a chronic wound or an ulcer.

19. Use of a material according to any of claims 1 to 14 to bind one or more cell growth factors.

20. A method of separating cell growth factors from a biological sample or organism, said method comprising:
(i) contacting said biological sample or organism with a material according to any of claims 1 to 14, the contacting being carried out *in vivo* or *in vitro*, to bind the growth

factors to the material; and
(ii) recovering the bound growth factors from the material.

21. A method of preparing an active wound dressing material comprising the steps of:
(i) contacting a material according to any of claims 1 to 14 with a biological medium containing cell growth factors to bind the cell growth factors to the material; and
(ii) washing and drying the material having the cell growth factors bound thereto to form said active wound dressing material.

22. A use according to claim 19, or a method according to claim 20 or 21, wherein the cell growth factors comprise platelet derived growth factor.

23. A process for the preparation of a wound dressing material, the process comprising the steps of:

providing an aqueous dispersion of a protein; immersing or dispersing oxidised regenerated cellulose in the aqueous dispersion; followed by

removing water from the aqueous dispersion to leave a material comprising the protein complexed with oxidised regenerated cellulose.

24. A process according to claim 23, wherein the protein and/or the oxidised regenerated cellulose are fibrous, substantially insoluble materials that form a suspension in water.

25. A process according to claim 23, wherein the oxidised regenerated cellulose is in the form of a knitted, woven or non-woven fabric that is immersed in the protein aqueous dispersion.

26. A process according to claim 23, 24 or 25, wherein the water is removed by freeze-drying or solvent drying to produce said material in the form of a sponge.

27. A process according to any of claims 23 to 26, wherein the aqueous dispersion contains 5-30mg/ml of the protein.

28. A process according to any of claims 23 to 27, further comprising the step of treating the protein in the dispersion, or in the material, with a cross-linking agent.

29. A process according to any of claims 23 to 28, wherein the pH of the protein dispersion is adjusted to pH 3-4.5.

30. A material or a process substantially as herein before described with reference to the examples.

The Patent Office

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Application No: GB 9613682.5
Claims searched: 1-30

Examiner: Nicola Curtis
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Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

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Other: ONLINE: WPI; BIOTECH/DIALOG

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
X	GB 2280850 A (Johnson & Johnson) (See page 7, lines 27-31; Example 2)	1-3,5,6, 8-15
X	EP 0049469 A1 (Dr. Ruhland Nachf) (See claim 1)	1,8,9,15,18 at least
X	CS 0269876 B1 (Stanislav et al.) (See WPI Abstract Accession No. 89-348621/48)	1,8,9,15 at least
X	WPI Abstract Accession No. 85-237601/39 & DE 3409372 A (Dr. Ruhland Nachf) (See abstract)	1,4,5,8,9, 15 at least
X	WPI Abstract Accession No. 85-155917/26 & JP 600087225 A (Unitika) (See abstract)	1,4,5,15, 18 at least
X	BIULL EKSP BIOL MED (USSR), Vol. 97, No. 7, 1981, Lekhtsind & Gurvich, "Synthesis of a high capacity immunosorbent based on a cellulose suspension", pages 68-70 and also MEDLINE Abstract Accession No. 82047152	1

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